Award Number: DAMD17-00-1-0366

TITLE: Miniaturized DNA Biosensor for Decentralized Breast-
            Cancer Screening

PRINCIPAL INVESTIGATOR: Joseph Wang, Ph.D.

CONTRACTING ORGANIZATION: New Mexico State University
                           Las Cruces, New Mexico 88003

REPORT DATE: June 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                        Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE June 2003	3. REPORT TYPE AND DATES COVERED Annual (1 Jun 02 – 31 May 03)

4. TITLE AND SUBTITLE
Miniaturized DNA Biosensor for Decentralized Breast-Cancer Screening

5. FUNDING NUMBERS
DAMD17-02-1-0239

6. AUTHOR(S)
Joseph Wang, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New Mexico State University
Las Cruces, New Mexico 88003

E-Mail: joewang@nmsu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The use of DNA testing as an important component of breast cancer diagnosis has been increasing rapidly over the past decade. The goal of this project is to develop and characterize an electrochemical biosensing microsystem for the rapid point-of-care genetic screening of breast-cancer. During the third year of this project we introduced new electrical routes for further improving the performance of DNA biosensors for genetic screening of breast-cancer. Particular attention has been given to simultaneous measurements of several breast-cancer related sequences and to the signal amplification in connection to several nanoparticle tracers and highly sensitive stripping voltammetric detection of the metal tag. Additional developmental work, aimed at transforming these powerful nanoparticle-based bioassays to a miniaturized flow system, is in progress towards the realization of wide-scale decentralized screening for breast cancer.

14. SUBJECT TERMS
Decentralized testing, electrochemical detection

15. NUMBER OF PAGES
25

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Introduction

Wide-scale genetic testing requires the development of fast, simple, miniaturized and inexpensive analytical devices. Traditional methods for detecting DNA hybridization are too slow and labor intensive. Biosensors offer a promising alternative for faster, simpler, and cheaper DNA assays. Hybridization biosensors commonly rely on the immobilization of a single-stranded (ss) oligonucleotide probe onto a transducer surface to recognize - by hybridization - its complimentary target sequence.

Electrochemical devices have received considerable attention in the development of sequence-specific DNA hybridization biosensors. Such devices rely on the conversion of the DNA base-pair recognition event into a useful electrical signal. The high sensitivity of such devices, coupled to their compatibility with modern microfabrication technologies, portability, minimal power requirements, low cost (disposability), and independence of optical pathway or sample turbidity, make them excellent candidates for decentralized DNA testing. Direct electrical reading of DNA hybridization thus offers great promise for developing simple, rapid, and easy-to-use, cost-effective DNA sensing devices (in a manner analogous to pocket-size blood-glucose meters).

The objective of this research is to develop and characterize a miniaturized biosensing system for decentralized genetic screening of breast-cancer. The realization of rapid point-of-care DNA testing requires proper attention to major challenges of high sensitivity and selectivity, multi-target detection, and integration of the sample preparation with the actual DNA detection on a single microchip flow platform. Such challenges are being met by designing innovative biosensor strategies that would allow testing for breast cancer to be performed more rapidly, inexpensively, and reliably in a decentralized setting.

Body: Progress Report

This report summarizes our activity over the third year of the project (i.e., the 7/02-6/03 period). In accordance to our original objectives our research focused on various fundamental and practical aspects of electrical detection of DNA segments specific to the breast-cancer gene BRCA1. As described in this section, we have made a substantial progress, and introduced innovative nanoparticle-based electrochemical routes for improving the reliability of devices for genetic screening of breast-cancer (based on new label-free and particle-based amplification schemes). This 11-mos activity has already resulted in 5 research papers (published or in press in major journals; see attached list) and several invited presentations in major meetings. (Several more publications are expected in the late part of 2003.) Such findings pave the way to major improvements in electrical detection of DNA and offer innovative routes for simple, rapid, and user-friendly devices for breast-cancer screening.

Signal Amplification for Detecting Low Target Concentrations. We examined several amplification processes for enhancing the sensitivity of our particle-based electrical DNA assays, including catalytic enlargement of the metal tracer, and use of polymeric microbeads carrying multiple redox tracers externally (on their surface) or internally (via encapsulation). Combined with additional amplification units and processes, such bead-

based multi-amplification protocols meet the high sensitivity demands of electrochemical DNA breast-cancer biosensors.

We have demonstrated a triple-amplification bioassay, coupling the carrier-sphere amplifying units (loaded with numerous gold nanoparticles tags) with the 'built-in' preconcentration of the electrochemical stripping detection and catalytic enlargement of the multiple gold-particle tags (1). The gold-tagged beads were prepared by binding biotinylated metal nanoparticles to streptavidin-coated polystyrene spheres. Such triple-amplification route offered a dramatic enhancement of the sensitivity, to allow detection of DNA targets down to the 300amol level. Factors affecting the performance have been optimized. Such amplified electrical transduction offers great promise for ultrasensitive detection of other biorecogntion events. An analogous amplification route, involving polymeric carrier spheres loaded with numerous CdS nanoparticles tags, is currently being examined in connection to the detection of cadmium.

Internal encapsulation electroactive tags within carrier beads offers another an attractive alternative to their external loading. For example, we developed an ultrasensitive electrical DNA detection based on polystyrene beads impregnated with a redox marker (2). The resulting 'electroactive beads' are capable of carrying a huge number of the ferrocenecarboxaldehyde marker molecules and hence offer a remarkable amplification of single hybridization events. This allowed chronopotentiometric detection of target DNA down to the 5.1×10^{-21} mol level (~31,000 molecules) in connection to 20 min hybridization and 'release' of the marker in an organic medium. The dramatic signal amplification advantage is combined with a remarkable discrimination against a huge excess (10^7) of non-complementary nucleic acids.

We also designed an electrochemical protocol for detecting DNA hybridization based on preparing the metal marker along the DNA backbone (instead of capturing it at the end of the duplex) (3). The new protocol relies on DNA-template induced generation of conducting nanowires as a mode of capturing the metal tag. The use of DNA as a metallization template has evoked substantial research activity directed to the generation of conductive nanowires and the construction of functional circuits. Yet, the DNA-templated assembly of metal wires has not been exploited for detecting DNA hybridization. The new detection scheme consists of the vectorial electrostatic 'collection' of silver ions along the captured DNA target, followed by hydroquinone-catalyzed reductive formation of silver aggregates along the DNA skeleton , along with dissolution and stripping detection of the nanoscale silver cluster. The new protocol thus combines the inherent signal amplification of stripping analysis with effective discrimination against nonhybridized DNA. It yielded a low detection limit of around 100ng/mL, which corresponds to 5 ng in the 50 μL hybridization solution. We have demonstrated for the first time the use of binary inorganic nanoparticles, such as CdS colloids, for electrochemical monitoring of DNA hybridization (4). The new protocol combines the amplification features of nanoparticle/polynucleotides assemblies and highly sensitive stripping potentiometric detection of cadmium, with an effective magnetic isolation of the duplex. The high sensitivity and selectivity make this protocol a useful addition to the armory of nanoparticle-based electrochemical genetic testing schemes. Other multi-amplification assays based on the use of dendritic highly-

branched ('tree-like') structures, bearing multiple metal nanoparticle tags are currently being developed.

Multi-Target DNA Detection: We designed an electrochemical coding technology for the simultaneous detection of multiple DNA targets based on nanocrystal tags with diverse redox potentials (5). Functionalizing the nanocrystal tags with thiolated oligonucleotide probes thus offered a voltammetric signature with distinct electrical hybridization signals for the corresponding DNA targets. The position and size of the resulting stripping peaks provided the desired identification and quantitative information, respectively, on a given target DNA. The multi-target DNA detection capability was coupled to the amplification feature of stripping voltammetry (to yield fmol detection limits) and with an efficient magnetic removal of nonhybridized nucleic acids to offer high sensitivity and selectivity. Up to 5-6 targets can thus be measured simultaneously in a single run in connection to ZnS, PbS, CdS, InAs, and GaAs semiconductor particles. Conducting massively parallel assays (in microwells of microtiter plates or using multi-channel microchips, with each microwell or channel carrying out multiple measurements) could thus lead to a high-throughput operation.

We demonstrated also a dual target electrochemical DNA detection based on the use of different enzyme tags (6). The two enzyme tracers selected for the initial demonstration of the dual-target detection, alkaline phosphatase (ALP) and β-galactosidase (GAL), liberate a wide range of phenolic products with different redox potentials. The dual target detection capability has been coupled with high sensitivity and effective discrimination against noncomplementary nucleic acids. The influence of relevant experimental variables was examined and optimized.

Key Research Accomplishments

During the third year of this project we introduced innovative electrochemical routes for improving the reliability of devices for genetic screening of breast-cancer. In particular, we have successfully designed new amplification protocol for enhancing the sensitivity of electrical devices and new coding protocols for simultaneous detection of multiple breast-cancer related sequences. The importance of these new bioassays is reflected from 5 publications in leading international journals. Such developments (combined with our early attention to the issues of mismatch discrimination or non-specific adsorption) should facilitate the realization of instant point-of-care breast-cancer testing. The successful realization of such testing will rely (during the 4th no-cost extension year) on the transformation of the new label-free and particle-based protocols into miniaturized flow systems (discussed in the Conclusions Section).

Reportable Outcomes

Papers submitted, accepted or published:

a. "Electroactive Beads For Ultrasensitive DNA Detection", J. Wang, R. Polsky, A. Merkoci, and K. Turner, Langmuir, 19(2003)989.

b. "Electrochemical Detection of DNA Hybridization based on DNA-Tempated Assembly of Silver Cluster", J. Wang, O. Rincon, R. Polsky, and E. Dominquez, Electrochem. Communications, 5(2003)83.

c. "Dual Enzyme Electrochemical Coding for Detecting DNA Hybridization", J. Wang, A. Kawde, M. Musameh and G. Rivas, Analyst 127(2002)1279.

d. "Electrochemical Stripping Detection of DNA Hybridization based on CdS Nanoparticle Tags", J. Wang, G. Liu, and R. Polsky, Electrochemistry Communications, 4(2002)819.

e. "Amplified Electrical Transduction of DNA Hybridization based on Polymeric Beads Loaded with Multiple Gold Nanoparticle tags", J. Wang and A. Kawde, submitted (to Langmuir, 2003).

<u>Invited Presentations</u>:

a. "Particle-based DNA Assays", Pittsburgh Conference (March 2003).

b. "Particle-based DNA Assay", Sensors 03, Paris (June 2003).

c. "Particle-based DNA Assay", Bioelectrochemistry, Florence (June 2003).

Conclusions

Electrochemical detection of DNA hybridization offers great promise for developing fast, simple, and user-friendly DNA sensing devices for point-of-care breast-cancer testing. By addressing the major challenge of signal amplification, our findings this year have led to major improvements in the sensitivity of electrical biosensing of DNA segments specific to the breast-cancer gene BRCA1. We also introduced novel electrical coding protocols for multi-target DNA detection. These new advances are coupled to our early schemes for minimizing non-specific adsorption and discriminating against non-complementary sequences.

The realization of decentralized DNA testing would require additional developmental work. Our coming efforts (during the no-cost extension period) will thus focus on transforming the new electrochemical protocols into microfluidic devices. The new microsystem will rely on the use of magnetic beads not only for removing unwanted constituents, but also for localizing different probes within the microchannels. The probe-bearing beads could thus be reproducibly introduced, kept in place, manipulated, and removed/replaced after each run (in connection to a proper placement and removal of a magnet). Such reversible magnetic localization obviates the need for 'fixing' the probe onto the chips, regenerating it, and for fabricating multiple hybridization sites or special physical 'barriers', and hence would greatly simplify the fabrication and operational requirements. The new microfluidic system will thus contain multiple functional elements and related microchannel network for integrating various sample-handling processes, including sample collection, DNA extraction, reagent mixing and amplification, with the actual hybridization detection. We have recently assembled a microfabrication laboratory that allows us to

micromachine the microfluidic devices essential for such chip-based high throughput automated operation. Preliminary fabrication of low-cost plastic microchips have been very successful. The coupling of these chip platforms with our particle-based DNA assays would accelerate the realization of wide-scale breast-cancer screening.

References

1. J. Wang and A. Kawde, submitted (to Langmuir 2003).

2. J. Wang, R. Polsky, A. Merkoci, and K. Turner, Langmuir, 19(2003)989.

3. J. Wang, O. Rincon, R. Polsky, and E. Dominquez, Electrochem. Communications, 5(2003)83.

4. J. Wang, G. Liu, and R. Polsky, Electrochemistry Communications, 4(2002)819.

5. J. Wang, G. Liu, and A. Merkoçi, J. Am. Chem. Soc., 125(2003)3214.

6. J. Wang, A. Kawde, M. Musameh and G. Rivas, Analyst 127(2002)1279.

Appendices

Papers resulted from our third year effort (as well as those published recently as a result of the second year funding):

Dual enzyme electrochemical coding for detecting DNA hybridization

Joseph Wang,* Abdel-Nasser Kawde, Mustafa Musameh and Gustavo Rivas†

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA. E-mail: joewang@nmsu.edu

Enzyme-based hybridization assays for the simultaneous electrochemical measurements of two DNA targets are described. Two encoding enzymes, alkaline phosphatase and β-galactosidase, are used to differentiate the signals of two DNA targets in connection to chronopotentiometric measurements of their electroactive phenol and α-naphthol products. These products yield well-defined and resolved peaks at +0.31 V (α-naphthol) and +0.63 V (phenol) at the graphite working electrode (vs. Ag/AgCl reference). The position and size of these peaks reflect the identity and level of the corresponding target. The dual target detection capability is coupled to the amplification feature of enzyme tags (to yield fmol detection limits) and with an efficient magnetic removal of non-hybridized nucleic acids. Proper attention is given to the choice of the substrates (for attaining well resolved peaks), to the activity of the enzymes (for obtaining similar sensitivities), and to the selection of the enzymes (for minimizing cross interferences). The new bioassay is illustrated for the simultaneous detection of two DNA sequences related to the BCRA1 breast-cancer gene in a single sample in connection to magnetic beads bearing the corresponding oligonucleotide probes. Prospects for electrochemical coding of multiple DNA targets are discussed.

Introduction

DNA hybridization assays have evolved dramatically during the past decade[1,2] Considerable recent attention has been given to the development of hybridization assays that permit simultaneous determination of two or more DNA targets in a single sample.[3–6] Multi-target hybridization assays, in which several targets are measured simultaneously in a single sample, represent several advantages and challenges. Such multiple sequence detection is highly desired, since, for example, a genetic disease can be caused by hundreds of different mutations. Most efforts in this direction have been devoted to simultaneous optical detection based on various optical coding avenues.[3–7] Mirkin's group[3] demonstrated recently that scattered light from different sized particle tags can be used for optical assays of multiple targets. Nie[4] reported on a multi-color coding based on embedding different quantum dots into microbeads. Keating and Natan[5] recently employed bar-coded microcods for the simultaneous analysis of multiple bioanalytes. Walt's team[6] described a fiber-optic DNA array based on different optically encoded microspheres. Unlike the considerable activity in developing optical coding for bioanalysis, little attention has been given to analogous electrochemical multi-tagging schemes.[7,8] It is thus highly desirable to develop new electrochemical coding technologies aimed at extending electrical DNA assays to the simultaneous analysis of multiple targets.

Here we report on a dual-enzyme electrochemical protocol for the simultaneous measurements of two DNA targets. As was

demonstrated for single-target DNA detection,[9,10] enzyme labels hold great potential for electrical detection of DNA hybridization, since their biocatalytic activity provides the amplification essential for monitoring low target levels. Several enzyme tags, generating electroactive products from electroinactive substrates, are readily adaptable to electrochemical bioassays.[11,12] In designing a multi-target electrical detection based on multiple enzyme tracers, it is essential to carefully consider the redox potential of the products of the biocatalytic reactions (and hence to avoid overlap of the electrochemical signals). Common potential windows of 1.2–1.4 V can accommodate 5–6 resolved peaks for multi-target DNA detection. The two enzyme tracers selected for the initial demonstration of the dual-target detection, alkaline phosphatase (ALP) and β-galactosidase (GAL), liberate a wide range of phenolic products with different redox potentials. The resulting electrochemical DNA hybridization sensing system couples an efficient magnetic isolation of the DNA duplexes, with the amplification provided by the two enzyme tags, and chronopotentiometric detection of the liberated phenol and α-naphthol products (Fig. 1). Such characteristics of the new dual-enzyme electrochemical coding protocol are reported in the following sections.

Experimental

Apparatus

Constant-current chronopotentiometry was performed with the TraceLab potentiometric unit (PSU20, Radiometer). Differential pulse voltammetry was carried out with an EG&G 264A voltammetric Analyzer connected to a BAS X-Y-t recorder. The preparation of the functionalized magnetic beads, the hybridization events and the biocatalytic reactions were performed
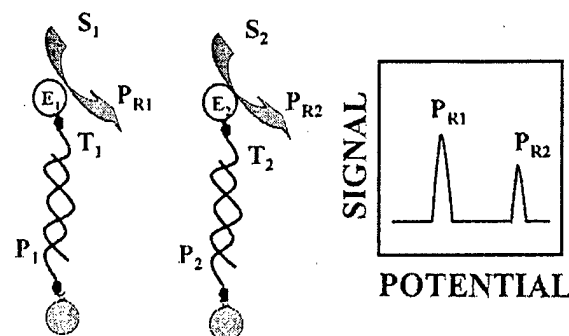

Fig. 1   Dual-enzyme electrochemical detection of two DNA targets. P_1: 5′-Biotinylated E908X-WT oligomer (Probe I); P_2: 5′-biotinylated 1675 delA-WT oligomer (Probe II); T_1 and T_2: DNA breast cancer targets; E_1 and E_2, the enzyme tags streptavidin–β-galactosidase (GAL) and streptavidin–alkaline phosphatase (ALP); S_1 and S_2 are the substrates (phenyl β-D-galactoside and α-naphthyl phosphate) and P_{R1} and P_{R2} are the products (phenol and α-naphthol) of the corresponding enzymatic reactions.

† Permanent address: Departamento de Fisico Quimica, Universidad Nacional de Cordoba, Cordoba, Argentina.

using a MCB 1200 Biomagnetic Processing Platform (Sigris Research Inc., Brea, CA).

Electrodes

A renewable graphite-pencil working electrode, similar to the one described earlier,[13] was used for detecting the enzymatically-liberated products. A Pentel pencil (Model AL25) was used for holding the 0.5 mm HB black graphite lead. Electrical contact was achieved by soldering a copper wire to the metallic part of the pencil holder. The pencil was fixed in a vertical position, exposing 11 mm of the lead (with 10 mm immersed in the solution). A platinum wire and Ag/AgCl served as the counter and reference electrodes, respectively.

Materials and reagents

All stock solutions were prepared using deionized and auto-claved water. Tween 20 was purchased from Aldrich. Tris-HCl buffer, LiCl, NaOH, NaCl, streptavidin–β-galactosidase (GAL; 387 U mg^{-1}), streptavidin–alkaline phosphatase (ALP; 760 U mg^{-1}), α-naphthol, α-naphthyl phosphate, phenol and phenyl β-D-galactoside were purchased from Sigma. Proactive streptavidin-coated microspheres (0.8 μm diameter, CM01N-Cat 4725) were purchased from Bangs Laboratories (Fishers, IN). Oligonucleotides with 5′-biotin modification were received from Life Technologies (Grand Island, NY). The corresponding sequences of the oligonucleotides are as follows:

5′-Biotinylated Probe I, E908X-WT (P1):
    5′-Biotin–GAT TTT CTT CCT TTT GTT C
5′-Biotinylated Target I, E908X-WT (T1):
    5′-Biotin–GAA CAA AAG GAA GAA AAT C
5′-Biotinylated Probe II, 1675 delA-WT (P2):
    5′-Biotin–TTT CTT GAT AAA ATC CTC A
5′-Biotinylated Target II, 1675 delA-WT (T2):
    5′-Biotin–TGA GGA TTT TAT CAA GAA A
5′-Biotinylated non-complementary target:
    5′-Biotin–5′-Biotin–CTT ACC CCA CCG ACC TCG G

Preparation of oligomer-coated microspheres and analytical procedure

The beads preparation was carried out using a modified procedure recommended by Bangs Laboratories.[14] Fifty micrograms of streptavidin-coated magnetic beads were transferred into two 1.5 ml centrifuge vials. The microspheres were washed with 90 μl TTL buffer (100 mM Tris-HCl pH 8.0, 0.1% Tween 20, 1 M LiCl) and resuspended in 21 μl of TTL buffer containing 4.0 μg of P$_1$ or P$_2$ to give final volumes of 25 μl. The probes were captured onto the beads during a 15 min incubation at room temperature with gentle mixing. Subsequently, 25 μl solutions of the two probe-functionalized magnetic beads were mixed in one vial, washed with 90 μl TTL buffer, and resuspended in 25 μl hybridization solution (750 mM NaCl and 150 mM sodium citrate, pH 8.5). At the same time, labeling of the two targets with the ALP and GAL enzyme tags was performed by adding the desired amount of the target to 0.5 ml vials containing either 1 μl streptavidin–β-galactosidase (1 U μl^{-1}) or 2 μl streptavidin–alkaline phosphatase (0.1 U μl^{-1}), and the required amount of TTL buffer to obtain final volumes of 12.5 μl. This was followed by a 15 min incubation at room temperature with gentle mixing. The solutions containing the two enzyme-labeled targets were then mixed into a single vial. The hybridization reaction was initiated by adding 25 μl of the β-galactosidase-labelled-T$_1$/alkaline phosphatase-labelled-T$_2$ mixture to the vial containing the magnetic beads bearing the P1 and P2 probes; the hybridization proceeded at room temperature with gentle mixing (usually for 10 min). The enzyme-labeled DNA conjugates were then washed twice with 90 μl TTL buffer

solution and resuspended in a 50 μl solution containing 50 mM of the corresponding substrates (phenyl β-D-galactoside and α-naphthyl phosphate). The samples were incubated at room temperature for 10 min to allow both enzymatic reactions to proceed. This was followed by magnetic separation and transfer of the 50 μl supernatant (containing the products of the enzymatic reactions) into the electrochemical cell containing 950 μl Tris-HCl buffer (0.5 M, pH 8.5).

Constant current chronopotentiometry was used for detecting the products of the enzymatic reactions. Each measurement was performed using a new 10 mm long graphite-lead surface. Such measurements were performed in a quiescent solution after a 5 s rest period using an initial potential of 0.0 V and an anodic current of +5.0 μA. The data were filtered and baseline-corrected with the TAP2 software.

Results and discussion

The new dual enzyme electrochemical coding protocol couples an efficient magnetic isolation of the DNA duplex, with the amplification provided by the two enzyme labels (β-galactosidase and alkaline phosphatase), and chronopotentiometric detection of the electroactive phenol and α-naphthol products (Fig. 1). The performance of the new bioassay is illustrated below for the simultaneous detection of two sequences related to the BCRA1 breast-cancer gene in connection to magnetic beads bearing the corresponding oligonucleotide probes. The use of a renewable graphite transducer obviates surface fouling problems associated with the anodic detection of the phenolic products.

GAL catalyzes the hydrolysis of the electroinactive β-D-galactosides while ALP catalyzes the reaction of electroinactive phenyl phosphates. Using an α-naphthyl-phosphate substrate of ALP and a phenyl-β-D-galactoside substrate of GAL thus results in α-naphthol and phenol products, respectively, whose electrochemical signals are well resolved. Fig. 2 displays differential-pulse voltammogram (A) and chronopotentiogram (B) for a sample mixture containing 20 μM of the α-naphthol (a) and phenol (b) products. Both compounds yield well-defined oxidation signals at the graphite pencil electrode, with anodic peaks at +0.31 V (α-naphthol) and +0.63 V (phenol). The efficient background correction capability of the chronopotentiometric operation results in sharper, baseline-resolved peaks. This detection method was thus used for all subsequent work. A constant current of +5 μA provided the most favorable compromise between high sensitivity and resolution and was used in all subsequent work.

In addition to the redox potentials of the reaction products, one must consider the attainment of similar sensitivities for the two DNA targets. This can be accomplished by adjusting the biocatalytic activity of the two enzyme tags or by controlling the substrate concentration. Adjusting the activity of the enzymes to 1.0 U GAL and 0.2 U ALP provided similar sensitivities (since
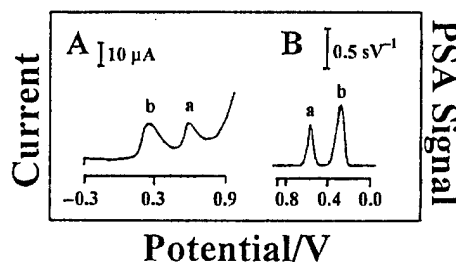

Fig. 2 Differential pulse voltammogram (A) and chronopotentiogram (B) for 20 μM α-naphthol (a) and 20 μM phenol (b) in a Tris-HCl (0.5 M, pH 8.5) solution. (A) Scan rate, 50 mV s^{-1}, amplitude, 50 mV; (B) constant current, + 5.0 μA.

the ALP reaction resulted in a 5-fold higher sensitivity than the GAL one when similar activities were used).

The dual enzyme electrochemical coding combines the high sensitivity of enzyme tagging with the efficient removal of non-hybridized DNA provided by the magnetic separation. Fig. 3 displays the response of the new bioassay to 1 µg l^{-1} of the E908X-WT (A) and 1675delA-WT (B) breast cancer DNA targets, in connection to their GAL and ALP tracers, respectively. Also shown (C) is the response to a large (200-fold) excess of a non-complementary nucleic acid. As expected (from the product signals of Fig. 2), the individual DNA targets yield well-defined chronopotentiometric peaks at +0.63 (A) and +0.31 V (B) corresponding to the oxidation of the enzymatically liberated phenol and α-naphthol, respectively. In contrast, no response is observed for the non-complementary DNA sequence (C), reflecting the effective magnetic separation.[15]

The position and size of the product peaks provide the desired identification and quantitative information, respectively, on a given target DNA. Such dual target quantitation is illustrated in Fig. 4A from the chronopotentiometric response to sample mixtures containing increasing levels of the two breast-cancer target oligonucleotides: 100 (1), 300 (2) and 500 (3) ng ml^{-1}. Well-defined and resolved peaks are observed, as needed for selective target identification; the peaks are proportional to the concentration of corresponding DNA targets, indicating minimal cross interferences and absence of surface fouling (associated with the use of renewable graphite electrodes). The resulting calibration plots are highly linear [Fig. 4B; correlation coefficients, 0.999(a) and 0.995(b)]. The very high sensitivity (associated with the enzymatic amplification) allows simultaneous monitoring of low levels of both targets. This is indicated from the response for a mixture containing 10 ng ml^{-1} of the two DNA targets (also shown in B). The favorable signal-to-noise (S/N) characteristics of these data indicate detection limits of ca. 0.7 (a) and 0.9 (b) ng ml^{-1} (i.e., 5.5 and 7.1 fmols in the 50 µl samples; S/N = 3). Even lower detection limits are expected in connection with enzyme/substrate recycling ampli-
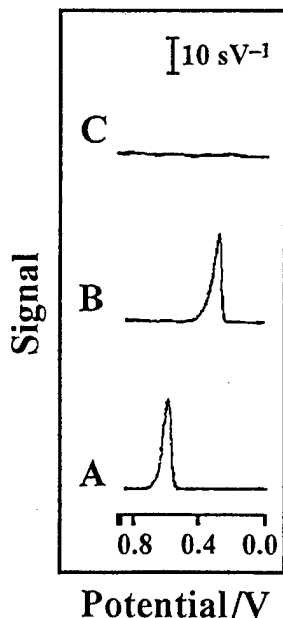

Fig. 4 (A) Chronopotentiometric signals for mixtures containing increasing levels of the E908X-WT and 1675delA-WT targets: (1) 100, (2) 300 and (3) 500 ng ml^{-1}. (B) The resulting calibration plots, as well as the response for a sample containing 10 ng ml^{-1} of the E908X-WT and 1675delA-WT targets. Other conditions, as in Fig. 2.

fication protocols.[16] The implementation of such amplification would require the absence of cross interferences.

Conclusions

We have demonstrated dual target electrochemical DNA detection based on the use of different enzyme tags. The dual target detection capability is coupled with high sensitivity and effective discrimination against non-complementary nucleic acids. This approach could be multiplexed and scaled by incorporating additional enzyme tracers and using multi-well plate or multi-channel chip formats. The multi-enzyme electrochemical coding could be adapted to other multi-analyte biological assays, particularly immunoassays. We are currently developing and comparing different electrochemical coding routes for extending electrical DNA assays to the simultaneous analysis of multiple DNA targets. Our goal is to increase the number of electrochemical codes that can be measured simultaneously, without overlap of their voltammetric signal. Up to 5–6 targets are expected to be measured simultaneously in a single run (with greater numbers when connected to parallel plate or chip operations). Particularly attractive in this direction is the use of 'electroactive' particles (i.e., polystyrene beads impregnated with different redox markers). Preliminary results in this direction are encouraging. The electrochemical coding technology is expected to open new opportunities for genetic testing in a manner similar to analogous optical multianalyte protocols.

Fig. 3 Chronopotentiometric hybridization response to: (A) 1 µg ml^{-1} E908X-WT (T$_1$), (B) 1 µg ml^{-1} 1675delA-WT (T$_2$), and (C) 200 µg ml^{-1} non-complementary (NC) oligomers; amount of magnetic beads, 50 µg; probe (P$_1$ or P$_2$) concentration, 160 µg ml^{-1}; streptavidin–β-galactosidase (GAL) activity, 1 Unit; streptavidin–alkaline phosphatase (ALP) activity, 0.2 Unit; phenyl β-D-galactoside (S$_1$) concentration, 50 mM; α-naphthyl phosphate (S$_2$) concentration, 50 mM; probe immobilization time, 15 min; enzyme association time, 15 min; hybridization time, 10 min; enzymatic reaction time, 10 min; constant current, +5 µA.
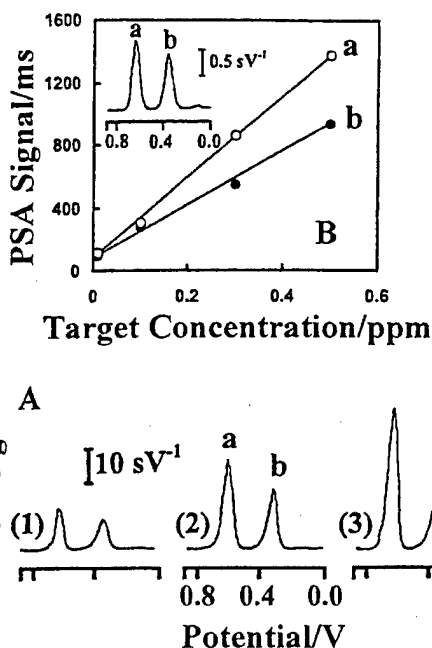
References

1 G. Ramsay, *Nature Biotechnol.*, 1998, **16**, 40.
2 J. Wang, *Nucleic Acids Res.*, 2000, **28**, 3011.
3 T. A. Taton, G. Lu and C. A. Mirkin, *J. Am. Chem. Soc.*, 2001, **123**, 5164.
4 M. Han, X. Gao, J. Su and S. Nie, *Nature Biotech.*, 2001, **19**, 631.
5 B. Reiss, L. He, I. Walton, R. Cromer, C. Keating and M. Natan, *Science*, 2001, **294**, 137.
6 J. A. Ferguson, F. J. Steemers and D. R. Walt, *Anal. Chem.*, 2000, **72**, 5618.
7 S. A. Brazil, P. Kim and W. G. Kuhr, *Anal.Chem.*, 2001, **73**, 4882.
8 I. Yang, P. Ropp and H. Thorp, *Anal. Chem.*, 2002, **74**, 347.
9 T. de Lumley, C. Campbell and A. Heller, *J. Am. Chem. Soc.*, 1996, **118**, 5504.
10 J. Wang, D. Xu, A. Erdem, R. Polsky and M. Salazar, *Talanta*, 2002, **56**, 931.
11 M. P. Kreunzer, C. K. O'Sullivan and G. G. Guilbault, *Anal. Chim. Acta*, 1999, **393**, 95.
12 W. R. Heineman and H. Halsall, *Anal. Chem.*, 1985, **57**, 31321A.
13 J. Wang and A. Kawde, *Anal. Chim. Acta*, 2000, **431**, 219.
14 Technote 101, Bangs Laboratories Inc., Fishers, IN.
15 E. Palecek, M. Fojta and F. Jelen, *Bioelectrochemistry*, 2002, **56**, 85.
16 F. W. Scheller, C. Bauer, A. Makower, U. Wolenberger, A. Warsinke and F. Bier, *Anal. Lett.*, 2001, **34**, 1233.

"Electroactive Beads" for Ultrasensitive DNA Detection

Joseph Wang, Ronen Polsky, Arben Merkoci, and
Kathryn L. Turner

Department of Chemistry and Biochemistry, New Mexico State
University, Las Cruces, New Mexico 88003, and Bangs Laboratories
Inc., Fishers, Indiana 46038

Langmuir®

The ACS Journal of Surfaces and Colloids

"Electroactive Beads" for Ultrasensitive DNA Detection

Joseph Wang,*,† Ronen Polsky,† Arben Merkoci,† and Kathryn L. Turner‡

Department of Chemistry and Biochemistry, New Mexico State University,
Las Cruces, New Mexico 88003, and Bangs Laboratories Inc., Fishers, Indiana 46038

Electrical transduction of DNA hybridization events is a major challenge in genoelectronics. Here we report on a new strategy for amplifying electrical DNA sensing based on the use of microsphere tags loaded internally with a redox marker. The resulting "electroactive beads" are capable of carrying a huge number of the ferrocenecarboxaldehyde marker molecules and hence offer a remarkable amplification of single hybridization events. This allows chronopotentiometric detection of target DNA down to the 5.1×10^{-21} mol level (~31 000 molecules) in connection to 20 min of hybridization and "release" of the marker in an organic medium. The dramatic signal amplification advantage is combined with a remarkable discrimination against a huge excess (10^7) of noncomplementary nucleic acids. Such electroactive beads hold great promise for multitarget detection (in connection to spheres loaded with different redox markers) and for enhancing the sensitivity of other bioassays.

Introduction

The detection of DNA hybridization is of central importance to the diagnosis and treatment of genetic diseases, for the detection of infectious agents, and for reliable forensic analysis. Such DNA sensing applications require high sensitivity through amplified transduction of the oligonucleotide interaction. Electrical detection of DNA hybridization has shown great promise for this purpose and has thus been the subject of intense research activity.[1] Such electronic transduction is commonly accomplished by using intercalating electroactive indicators (that associate with the surface hybrid),[2] through enzyme-amplified recognition,[3] by monitoring the intrinsic redox activity of DNA,[4] or through redox tags covalently bound to single-stranded DNA oligomers.[5] In particular, the use of ferrocene–oligonucleotide conjugates has been shown to be extremely useful for monitoring DNA and RNA down to the femtomole level.[5] Here we report on a dramatic amplification of single hybridization events based on the use of novel "electroactive beads" that results in a 1000-fold sensitivity enhancement over previous electronic sensors.

Particle-based materials offer excellent prospects for DNA analysis.[6] The new amplification strategy relies on the use of polystyrene microbead tags which are internally "loaded" with organic media-soluble electroactive markers in a manner analogous to the entrapment of fluorescent agents in commercial "fluorescent microspheres".[7] The resulting electroactive beads are capable of carrying a huge number of marker molecules and hence offer a dramatic amplification of single hybridization events and remarkably low detection limits (down to 5.1×10^{-21} mol or ~31 000 molecules). Such beads are prepared by a diffusion/entrapment process in the presence of the marker/organic-solvent solution, in a manner similar to the preparation of their fluorescent analogues.[7a] Approximately 5×10^{11} molecules of the ferrocenecarboxaldehyde (FCA) marker have thus been encapsulated in a single microsphere (see Experimental Section). Such internal loading affords the availability of neutravidin surface groups for DNA binding. The huge signal amplification is coupled to an effective discrimination against noncomplementary nucleic acids.

Experimental Section

Chronopotentiometric measurements were performed with a potentiometric stripping unit PSU20 (Radiometer) controlled by the TAP2 software (Radiometer), and a 1.0 mL cell containing the acetonitrile/0.2 M tetraethylammonium chloride solution. The preparation of the probe-coated magnetic beads and the hybridization reaction were performed on a MCB 1200 Biomagnetic Processing Platform (Sigris). An IEC Micromax centrifuge (OM3590) and a Vortex Genie2 shaker were used during the ferrocenecarboxaldehyde bead labeling of the target DNA. Beads (magnetic and FCA loaded) were received from Bangs Laboratories Inc. and had binding capacities of 0.90 and 0.009 μg of biotin–FITC mg^{-1} of microspheres, respectively. Buffers and stock solutions were prepared from deionized and autoclaved water.

The FCA loading capacity (5×10^{11} molecules/sphere) was estimated from measurements done on 7000 FCA-doped microspheres which yielded an electrochemical response similar to that of a 6.6×10^{-6} M, 1 mL FCA solution.

The probe immobilization onto the magnetic beads was performed with the MCB 1200 Biomagnetic Processing Platform using a modified procedure recommended by Bangs Laboratories (Technote 101). Briefly, 50 μg of streptavidin-coated microspheres was transferred into a 1.5 mL centrifuge tube. The microspheres

---

* To whom the correspondence should be addressed. E-mail: joewang@nmsu.edu.
† New Mexico State University.
‡ Bangs Laboratories Inc..

(1) (a) Mikkelsen, S. R. *Electroanalysis* **1996,** *8,* 15. (b) Wang, J. *Chem. Eur. J.* **1999,** *5,* 1681. (c) Palecek, E.; Fojta, M. *Anal. Chem.* **2001,** *73,* 75A.

(2) (a) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chem.* **1994,** *66,* 3830. (b) Millan, K. M.; Saraullo, O.; Mikkelsen, S. R. *Anal. Chem.* **1994,** *66,* 2943.

(3) (a) Lumley-Woodyear, T. D.; Campbell, C. N.; Heller, A. *J. Am. Chem. Soc.* **1996,** *118,* 5504. (b) Patolsky, F.; Katz, E.; Bardea, A.; Willner, I. *Langmuir* **1999,** *15,* 3703.

(4) (a) Johnston, D. H.; Glasgow, K.; Thorp, H. H. *J. Am. Chem. Soc.* **1995,** *117,* 8933. (b) Wang, J.; Nasser, A.; Erdem, A.; Salazar, M. *Analyst* **2001,** *126,* 2020.

(5) (a) Ihara, T.; Nakayma, M.; Murata, M.; Nakano, K.; Maeda, M. *Chem. Commun.* **1997,** 1609. (b) Nakayama, M.; Ihara, T.; Nakano, K.; Maeda, M. *Talanta* **2002,** *56,* 857. (c) Yu, C. J.; Wan, Y.; Yowanto, H.; Li, J.; Tao, C.; James, M. D.; Tan, C.; Blackburin, G.; Meade, T. *J. Am. Chem. Soc.* **2001,** *123,* 11155.

(6) (a) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998,** *120,* 1959. (b) Wang, J.; Xu, D.; Kawde, A. N.; Polsky, R. *Anal. Chem.* **2001,** *73,* 5576. (c) Patolsky, F.; Ranjit, K. T.; Lichtenstein, A.; Willner, I. *Chem. Commun.* **2000,** 1025.

(7) (a) Technote 103, Bangs Laboratories Inc., Fishers, IN, 2000 (www.bangslabs.com). (b) Bellisaro, R.; Colinas, R. J.; Pass, K. A. *Clin. Chem.* **2000,** *46,* 1422.
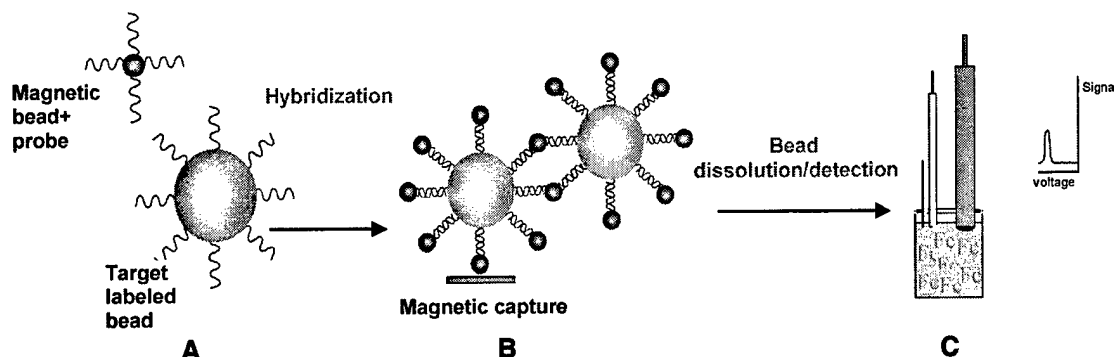
Figure 1. Steps involved in the new genoelectronic protocol: (A) Introduction of probe-coated magnetic beads and target-labeled electroactive beads. (B) Hybridization and magnetic separation. (C) Dissolution of the spheres in acetonitrile, release of the marker, and its chronopotentiometric detection at a glassy carbon electrode. Fc represents the ferrocene marker.

were washed with 100 μL of TTL buffer (100 mM Tris-HCl, pH 8.0, and 0.1% Tween 20, 1 M LiCl) and resuspended in 20 μL of TTL buffer. Subsequently, 4.0 μg of the biotinylated probe was added and incubated for 15 min at room temperature with gentle mixing. The coated beads were then separated and washed twice with 100 μL of TT buffer (250 mM Tris-HCl, pH 8.0, and 0.1% Tween 20) and resuspended in 20 μL of hybridization buffer (750 mM NaCl, 150 mM sodium citrate). The appropriate amount of the biotinylated target (or noncomplementary) nucleic acid was added to 40 μL of neutravidin-coated electroactive beads (18.73 × 10^6 particles mL^{-1}) in TTL buffer and incubated for 30 min with gentle mixing, washed with 100 μL of TT buffer, centrifuged at 8000 rpm for 2 min, and resuspended in 30 μL of the hybridization buffer. The hybridization event proceeded by gently mixing for 20 min in the bead-coated probe and target solutions (50 μL total volume). This was followed by washing twice with 90 μL of TT buffer and resuspending the conjugate in 20 μL of TT buffer. During the decanting step special attention must be given, as a small fraction of the DNA−particle assembly tends to fall below the magnetic field. The resulting 20 μL sample was then spiked into the 1 mL of acetonitrile (containing 0.2 M tetraethylammonium chloride) dissolution/detection solution, where the spheres were dissolved for 5 min under gentle stirring. Chronopotentiometric detection of the released FCA was carried out at a glassy carbon electrode (polished with a 0.05 μm alumina slurry) using a constant current of +2 μA (following 5 s at +0.20V), and in connection to Ag/AgCl and platinum-wire reference and counter electrodes, respectively.

All DNA oligonuclotides were purchased from Sigma-Genosys Ltd. The oligonucleotides had the following sequences: probe, 5′-biotin-GTA TTA GCT TTT CTT GAT AAA ATC CTC A; target, 5′-biotin-GTA TTA GCT TGA GGA TTT TAT CAA GAA A; noncomplementary, 5′-biotin-GTA TTA GCT GAA CAA AAG GAA GAA AAT C; 2-bp mismatched oligomer sequence, 5′-biotin-GTA TTA GCT T̲CA G̲CA TTT TAT CAA GAA A.

Results and Discussion

Figure 1 outlines the steps of the new microsphere-based bioelectronic protocol. DNA probe-coated magnetic beads are introduced to a 50 μL hybridization solution along with the electroactive-bead tagged complementary DNA (A). Following a 20 min hybridization and magnetic separation (B), the resulting magnetic-bead/DNA/FCA-microsphere conjugates are transferred into an acetonitrile solution, which triggers the release of the marker (in connection to dissolution of the beads). This is followed by a chronopotentiometric detection of the released marker at a glassy carbon transducer (C). (Practical applications would involve sandwich or competitive assays without labeling the target.)

Figure 2 displays a scanning electron micrograph of the DNA-linked particle assembly, which resulted from the hybridization event. This image indicates that the 10 μm electroactive beads are cross-linked to the smaller (~0.8 μm) magnetic spheres through the DNA hybrid.
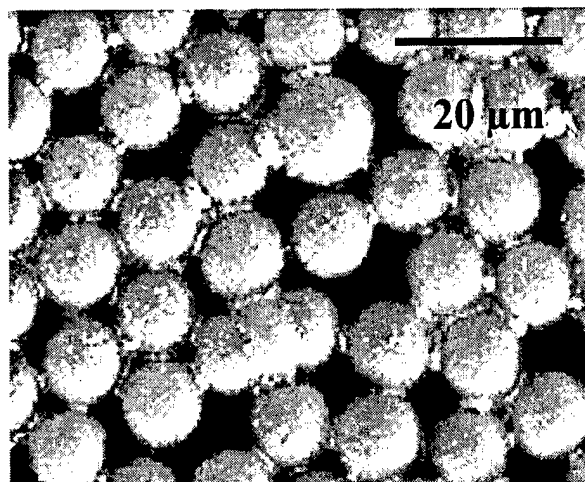

Figure 2. Scanning electron micrograph of a DNA−particle assembly produced following a 20 min hybridization with a 40 mg L^{-1} target. The micrograph was taken on a Hitachi S3200 instrument.

Such hybridization-induced aggregation is common to particle-based DNA assays.[6] The image indicates also that the integrity of the particle-linked DNA network is maintained during the washing and magnetic-separation steps. Similar particle-linked DNA networks were observed using electroactive beads of 5 and 20 μm diameter (not shown). In contrast, such aggregation was not observed in the presence of noncomplementary DNA; a 2-bp mismatched oligonucleotide resulted in greatly smaller aggregates (not shown).

Electrical detection of DNA segments related to the BRCA1 breast cancer gene was used for illustrating a remarkably enhanced signal per hybridization reaction. Figure 3 displays typical chronopotentiograms for extremely low target concentrations (10−100 000 pg L^{-1}; A−C), along with the corresponding response for a huge (~10^3-fold to 10^7-fold) excess of a noncomplementary nucleic acid (D). Well-defined oxidation peaks are observed for the low target levels (E_p = +0.88 V). The favorable response of the 10 pg L^{-1} DNA target (A) indicates a remarkably low detection limit of 1 pg L^{-1} (~10^{-16} M) in connection to the 20-min hybridization time. Such a detection limit corresponds to 5.1 × 10^{-21} mol (i.e., ~31 000 molecules) in the 50 μL sample and represents the lowest value reported for electrical DNA detection. The new detection system is 10^6 and 10^3 times more sensitive than analogous DNA sensing based on ferrocene-conjugated oligonucleotides[5] or the most sensitive electrical assay
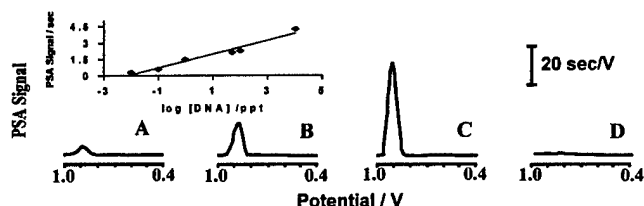
Figure 3. Chronopotentiometric hybridization signals for 0.01 (A), 0.1 (B), and 100 (C) ng L^{-1} (ppt) DNA targets and 500 μg L^{-1} (ppb) noncomplementary DNA (D). Inset: calibration plot for DNA target concentrations ranging from 0.01 to 10 000 ng L^{-1}. Hybridization time, 20 min in 0.75 M NaCl/0.15 M sodium citrate; marker release time, 5 min; constant current, 2 μA. Measurements were carried out with a Trace Lab PSU20 system (Radiometer), controlled by the TAP2 software, and a 1.0 mL cell containing the acetonitrile/0.2 M tetraethylammonium chloride solution.

based on liposome amplification,[8] respectively. A negligible signal is observed for the 500 μg L^{-1} noncomplementary DNA (D). Apparently, the combination of effective magnetic separation and transfer to an organic detection medium eliminates nonspecific adsorption and contributions of nonhybridized DNA. A 1000 ng L^{-1} 2-bp mismatch solution yielded a small response, corresponding to that of a 0.1 ng L^{-1} target (not shown).

The above target measurements are part of a six-point calibration experiment over the 0.01−10 000 ng L^{-1} range. The peak area increased nonlinearly with the target concentration. Yet, the resulting logarithmic plot (shown in Figure 3) is linear over the entire range and is suitable for quantitative work. Similar profiles were reported for gold-nanoparticle bioassays.[6b,9] Saturation of the probe hybridization sites and changes in the degree of

aggregation account for the nonlinear concentration dependence.[6b,9,10] Similar models of colloidal aggregation due to avidin/biotin systems predict a distinct transition between limited and complete aggregation as a function of ligand (DNA) to receptor (polymer bead) ratio.[10] The amplified signal is coupled to a relatively good reproducibility (e.g., a RSD of 23% for five successive measurements of the 100 pg L^{-1} target). Such signal variations reflect primarily the reproducibility of the aggregation process, associated with the consistency of the bead preparation and labeling steps.

In conclusion, we have demonstrated the use of redox-marker encapsulated polystyrene microspheres for dramatically amplifying DNA sensing processes. The signal amplification advantage has been combined with an efficient magnetic removal of noncomplementary DNA. While the use of electroactive beads has been presented in connection to the detection of DNA hybridization, it can be readily extended to the monitoring of other biorecognition events (e.g., immunochemical reactions). While the current protocol has relied on labeled targets, practical applications would involve sandwich or competitive assays. Current efforts in our laboratories are aimed at the simultaneous detection of mixed target solutions based on microspheres loaded with different markers (with a range of redox potentials). We are also examining a further signal amplification through surface accumulation of ferrocenyl ions onto ion-exchanger modified electrodes.

(8) Patolsky, F.; Lichtenstein, A.; Willner, I. *Angew Chem., Int. Ed.* **2000,** *39,* 940.
(9) Thanh, N. T.; Rozenzwieg, Z. *Anal. Chem.* **2002,** *74,* 1624.

(10) Kisak, E. T.; Kennedy, M. T.; Trommeshauser, D.; Zasadinski, J. A. *Langmuir* **2000,** *16,* 2825.

ELSEVIER

Electrochemical stripping detection of DNA hybridization based on cadmium sulfide nanoparticle tags

Joseph Wang *, Guodong Liu, Ronen Polsky, Arben Merkoçi

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA

Received 9 July 2002; accepted 22 July 2002

Abstract

We report on the detection of DNA hybridization in connection to cadmium sulfide nanoparticle tracers and electrochemical stripping measurements of the cadmium. A nanoparticle-promoted cadmium precipitation is used to enlarge the nanoparticle tag and amplify the stripping DNA hybridization signal. In addition to measurements of the dissolved cadmium ion we demonstrate solid-state measurements following a 'magnetic' collection of the magnetic-bead/DNA-hybrid/CdS-tracer assembly onto a thick-film electrode transducer. The new protocol combines the amplification features of nanoparticle/polynucleotides assemblies and highly sensitive stripping potentiometric detection of cadmium, with an effective magnetic isolation of the duplex. The low detection limit (100 fmol) is coupled to good reproducibility (RSD = 6%). Prospects for using binary inorganic colloids for multi-target detection are discussed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Nanoparticles; DNA hybridization; Cadmium sulfide; Stripping potentiometry

1. Introduction

The development of DNA biosensors has been the subject of an intense activity [1–3]. Such detection of DNA hybridization has greatly benefited from the recent use of nanoparticle materials [4–9]. The unique properties of nanometer-sized materials make them excellent candidates for DNA sensing [11]. Recent activity in this area has involved the use of gold or silver nanoparticle probes for optical [4,5] or electrochemical [6–8] monitoring of DNA hybridization, and the use of quantum-dot tagging for optical [9] or photoelectrochemical [10] gene sensing.

Here we report on a nanoparticle-based electrochemical detection of DNA hybridization based on cadmium sulfide (CdS) nanoparticle tags and potentiometric stripping measurements of the dissolved cadmium tracer. The use of metal labels (e.g., Au, Ag) for sensing DNA hybridization in connection to highly sensitive stripping detection has been demonstrated [6–8]. Combining the catalytic enlargement of the metal-particle tags, with the effective 'built-in' amplification of electrochemical stripping analysis, has thus paved the way to remarkably low (fmol) detection limits of target DNA. Analogous protocols based on binary inorganic colloids have not been reported, despite the rich chemistry of such inorganic nanoparticles. Particularly attractive for such bioassays are CdS and PbS semiconductor particles in view of the attractive stripping behavior of cadmium or lead ions [12]. Fig. 1 outlines the steps of the new bioelectronic protocol, including the binding of the biotinylated target to streptavidin-coated magnetic beads (A), its hybridization event to a CdS-labeled probe (B), dissolution of the CdS tag (C) and stripping potentiometric detection of the dissolved cadmium ion (D). We will also demonstrate that the dissolution step (C) can be eliminated and that solid-state detection of the cadmium can be accomplished through 'magnetic' collection of the DNA-linked nanoparticle network [13]. The performance characteristics of the new CdS-nanoparticle based nucleic-acid detection protocol are reported in the following sections.

---
* Corresponding author. Tel.: 1-505-646-2140; fax: 1-505-646-6033.
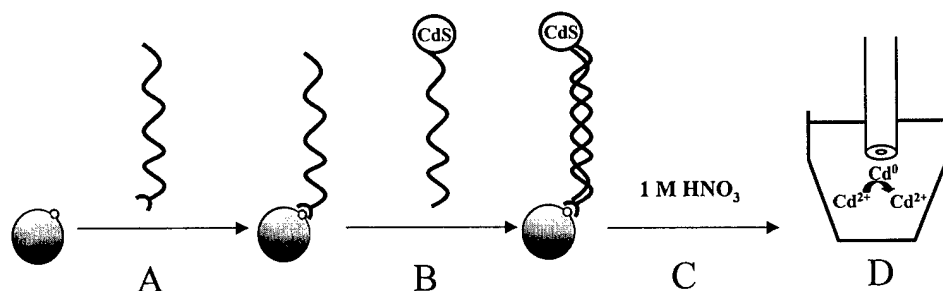 *E-mail address:* joewang@nmsu.edu (J. Wang).

Fig. 1. Schematic representation of the analytical protocol: (A) binding of the target to the magnetic beads; (B) hybridization with the CdS-labeled probe; (C) dissolution of CdS tag; (D) potentiometric stripping detection at a mercury-film electrode.

2. Experimental

2.1. Apparatus

Chronopotentiometric measurements were performed with a potentiometric stripping PSU20 system (Radiometer), controlled by a PC using the TAP2 software (Radiometer). The detection was carried out in a 1.5 ml electrochemical cell containing a mercury-coated glassy carbon disk working electrode (2 mm diameter), an Ag/AgCl reference electrode (CH Instruments, Austin, TX), and a platinum wire counter electrode. The magnetic bead assays were performed on an MCB 1200 Biomagnetic Processing Platform (Dexter, CA, USA). The setup for the magnetic 'collection' experiments was described elsewhere [13].

2.2. Reagents

All stock solutions were prepared using deionized and autoclaved water. The sodium acetate buffer (3 M, pH 5.2), nitric acid, cadmium nitrate, sodium sulfide, sodium bis(2-ethylhexyl)sulfosuccinate(AOT), pyridine, n-heptane, acetone, methanol, cystamine, 2-sulfanylethane sulfonic acid, Tris–HCl buffer, hydroquinone, lithium chloride and sodium chloride were purchased from Sigma. Tween 20 was purchased from Aldrich. Proactive streptavidin-coated microspheres (CMO1N) were purchased from Bangs Laboratories.

The DNA oligonucleotides were obtained from Life Technologies (Grand Island, NY, USA) and had the following sequences:

Target: biotin-5'ATAGACTGGCCGTCGTTTTAC AACGTCGTGGT

Probe: 5'-SH-(CH₂)₆-CCACGACGTTGTAAAACG ACGGCCAGTCTAT

Non-complementary: biotin-5'CAAAACGTATTTT GTACAAT

2.3. Preparation of CdS nanoparticles

Cadmium sulfide nanoparticles were prepared based on a slightly modified literature protocol [10]. Briefly, an

AOT/n-heptane water-in-oil microemulsion was prepared by the solubilization of 4 ml of distilled water in 200 ml n-heptane in the presence of 14 g of AOT surfactant. The resulting mixture was separated into 120 and 80 ml reverse-micelle subvolumes. Then, 0.48 ml of 1.0 M $Cd(NO_3)_2$ solution and 0.32 ml of 1.0 M Na_2S solution were added into the 120 and 80 ml subvolumes of reverse micelles, respectively. After stirring the two subvolumes for 1 h, they were mixed and stirred under helium for 1 h to yield the CdS nanoparticles. Subsequently, 0.34 ml of a 0.32 M cystamine solution and 0.66 ml of 0.32 M 2-sulfanylethane sulfonic acid were added; the resulting mixture was stirred for 24 h under helium. It was then evaporated under vacuum and the residue was successively washed with pyridine, n-heptane, acetone, and methanol to yield the cystamine/thioethanesulfonate-capped, water-soluble CdS nanoparticles.

2.4. Preparation of CdS–DNA conjugate

An aqueous solution of the CdS nanoparticles (1 mg ml⁻¹) was prepared and was than exposed to the thiolated oligonucleotide probe (8 OD ml⁻¹). The mixture was stirred for 24 h at room temperature under helium and was gradually brought to 0.24 M NaCl and 0.1M phosphate buffer (pH 7.4). The resulting solution was dialyzed for 48 h against 0.2 M NaCl and 0.1 M phosphate buffer (pH 7.4) containing 0.01% sodium azide.

2.5. Preparation of oligonucleotide-coated magnetic beads and the analytical procedure

The target modified magnetic beads were prepared in a MCB 1200 Biomagnetic Processing Platform using a modified procedure recommended by Bangs Laboratories (Tech Note 101). Briefly, 2 μl streptavidin-coated magnetic beads were transferred into a 1.5 ml centrifuge tube. The beads were then washed with 95 μl TTL buffer (100 mM Tris–HCl, pH 8.0, 0.1% Tween, and 1 M LiCl) and suspended in 21 μl TTL buffer. Then, 4 μl of the corresponding biotinylated target were added and incubated for 15 min with gentle mixing (Fig. 1(A)). The beads were subsequently washed twice with 95 μl TT

buffer (250 mM Tris–HCl and 0.1% Tween 20) and suspended in 45 µl hybridization buffer (750 mM NaCl, 150 mM sodium citrate). Then, 5 µl CdS–DNA target was added and mixed for 10 min (Fig. 1(B)). The resulting hybrid-conjugated microspheres were then washed twice with 95 µl TT and resuspended in 25 µl of 1 M HNO_3 solution. Dissolution of the CdS tag (Fig. 1(C)) thus proceeded for 3 min using magnetic stirring. Following a magnetic separation, the 25 µl HNO_3 solution (containing the dissolved Cd^{2+}) was transferred into 1 ml acetate buffer (pH 5.2). Chronopotentiometric stripping measurements of the dissolved cadmium ion were performed at a mercury-film electrode using a 2 min deposition at −0.90 V in a stirred acetate buffer solution (0.2 M, pH 5.2; 1 ml). Subsequent stripping was carried out after a 10 s rest period (without stirring) using an anodic current of +1.0 µA. The stripping curve data were filtered and baseline corrected using the TAP2 software. The mercury-film electrode was prepared on a polished glassy carbon electrode by applying a potential of −1.10 V for 10 min using a 0.1 M HCl solution containing 100 mg l^{-1} Hg^{2+}.

2.6. Particle enlargement and magnetic 'collection' protocols

Cadmium catalytic precipitation experiments were performed by a 20 min incubation of the sample (following the hybridization) in a solution containing 30 µl of 0.05 M $Cd(NO_3)_2$ and 30 µl of 0.05 M hydroquinone. 'Magnetic' collection experiments were conducted using a mercury-coated screen-printed carbon electrode (prepared by 10 min deposition at −1.10 V in a 0.1 M HCl solution containing 100 mg l^{-1} Hg^{2+}) by placing a magnet directly under the working electrode to anchor the particle-DNA assembly before placing the 60 µl DNA-particle aggregate sample for measurements (vs. Ag/AgCl) in a manner analogous to that described elsewhere [13].

3. Results and discussion

The new procedure couples the high sensitivity of nanoparticle-based stripping assays with a magnetic discrimination against non-complementary nucleic acids. The present protocol relies on binary inorganic (CdS) nanoparticles, compared to single (Au or Ag) metal colloids used in early stripping DNA procedures [6–8,14]. Fig. 2 displays typical chronopotentiograms for solutions containing increasing target concentrations in 200 ng ml^{-1} steps (A–C), as well as to a large excess of non-complementary oligonucleotide (D). Well-defined cadmium stripping peaks ($E_p = -0.52$ V) are observed in connection to these low target concentrations. The cadmium signals are proportional to the target concen-
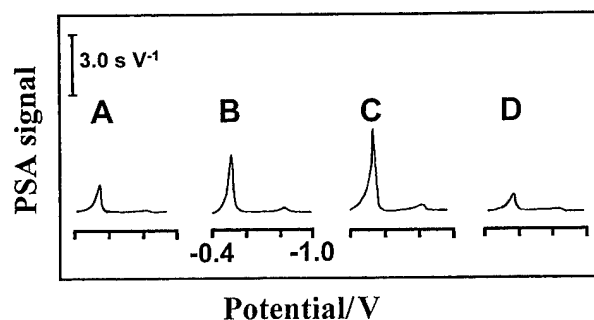

Fig. 2. Stripping potentiograms for increasing DNA target concentrations in 0.2 mg l^{-1} steps (A–C), as well as for a 6.0 mg l^{-1} non-complementary solution (D). Amount of beads, 20 µg; concentration of probe modified with CdS nanoparticles, 0.01 mg ml^{-1}; hybridization time, 10 min; accumulation potential, −0.90 V; accumulation time, 2 min; stripping current, 1 µA.

tration. A substantially smaller signal is observed for an excess (6 µg ml^{-1}) of the non-complementary DNA. Such minimization of non-specific binding is attributed to the efficient magnetic separation, i.e., removal of non-hybridized DNA. Repeated measurements using a 1.0 µg ml^{-1} target DNA solution yielded reproducible signals with a relative standard deviation of 6.0% (not shown; $n = 6$).

In addition to effective discrimination against non-hybridized DNA [15], the use of magnetic beads can offer additional advantages. For example, a substantial simplification of such nanoparticle-based electrical assays has been realized recently in our laboratory in connection to a 'magnetic' collection of the cross-linked DNA-particle assembly onto a thick-film electrode transducer to allow direct electrical contact of the silver precipitate [13]. A similar solid-state electrochemical detection can be achieved using the CdS-based DNA assemblies. Such assemblies can be magnetically anchored onto the film electrode to facilitate the anodic dissolution of the cadmium. Fig. 3 compares cadmium stripping potentiograms obtained for a 1.0 µg ml^{-1} target DNA using solution-phase (A) and solid-state (B) measurements of the cadmium tracer. Similar stripping peaks are observed in both cases, reflecting the efficiency of the solid-state detection (i.e., the good CdS-surface contact). This obviates the need for the acid dissolution of the cadmium tracer.

Further amplification of the sensitivity of the present nanoparticle-based DNA electrochemical detection can be achieved by catalytic precipitation of cadmium to produce larger tagging particles. Fig. 4 compares the stripping hybridization response for a 400 ng ml^{-1} target solution in the absence (A) and presence (B) of the cadmium precipitation step. A large (>12 fold) enhancement of the cadmium hybridization signal is observed following a 20-min precipitation, reflecting the substantial particle enlargement. Analogous enlargement of the gold metal tags and amplification of the
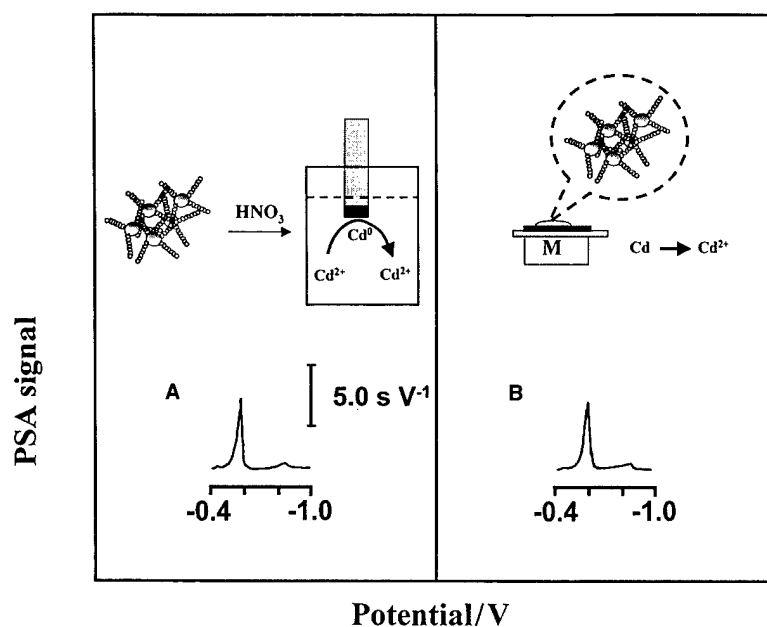
Potential/V

Fig. 3. Stripping potentiograms for 1 mg l^{-1} target, following (A) acid dissolution and electrodeposition and in connection (B) to magnetic 'collection' and solid-state detection. Other conditions, as Fig. 2.
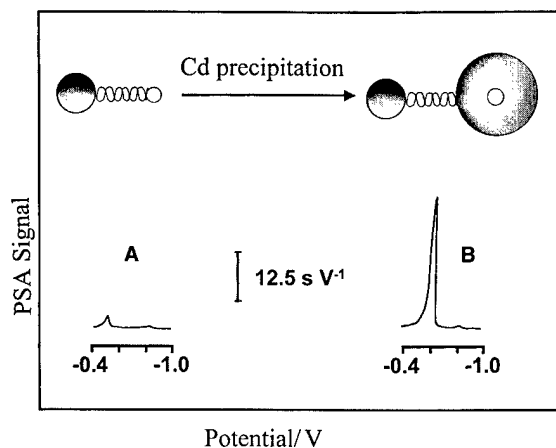

Potential/V

Fig. 4. Stripping potentiograms for 0.4 mg l^{-1} target, without (A) and with (B) catalytic enhancement with 30 µl of 10 mM Cd(NO$_3$)$_2$ and 30 µl of 0.05 M hydroquinone. Other conditions, as Fig. 2.

stripping DNA hybridization signal has been reported [6]. A detection limit of 20 ng ml^{-1} DNA target (corresponding to 100 fmol in the 50 µl sample) can thus be estimated. Even lower detection limits are expected in connection to longer hybridization periods and/or deposition times.

4. Conclusions

We have demonstrated for the first time the use of binary inorganic nanoparticles, such as CdS colloids, for electrochemical monitoring of DNA hybridization. The high sensitivity and selectivity make this protocol a useful addition to the armory of nanoparticle-based electrochemical genetic testing schemes. While the concept has been presented within the context of CdS particles, it could be readily extended to other inorganic colloids (e.g., ZnS or PbS) that can be similarly synthesized in reversed micelles [16]. Such extension should pave the way to the simultaneous analysis of several DNA targets in a manner analogous to the multicolor coding of Nie and co-workers [9]. Another promising route could involve the 'collection' of positively charged CdS particles along the DNA duplex in a manner analogous to the preparation of nanoparticle wires [17].

References

[1] J. Wang, Nucleic Acids Res. 28 (2000) 3011.
[2] E. Palecek, M. Fojta, Anal. Chem. 73 (2001) 75A.
[3] G. Ramsay, Nat. Biotechnol. 16 (1998) 40.
[4] J.J. Storhoff, R. Elghanian, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 120 (1998) 1959.
[5] T.A. Taton, C.A. Mirkin, R.L. Letsinger, Science 289 (2000) 1757.
[6] J. Wang, D. Xu, A.N. Kawde, R. Polsky, Anal. Chem. 73 (2001) 5576.
[7] J. Wang, R. Polsky, D. Xu, Langmuir 17 (2001) 5739.

[8] L. Authier, C. Grossiord, P. Berssier, B. Limoges, Anal. Chem. 73 (2001) 4450.

[9] M. Han, X. Gao, J.Z. Su, S. Nie, Nat. Biotech. 19 (2001) 631.

[10] I. Willner, F. Patolsky, J. Wasserman, Angew. Chem. Int. Ed. 40 (2001) 1861.

[11] C.M. Niemeyer, Angew. Chem. Int. Ed. 40 (2001) 4129.

[12] J. Wang, Stripping Analysis, VCH, New York, 1985.

[13] J. Wang, D. Xu, R. Polsky, R. J. Am. Chem. Soc. 124 (2002) 4208.

[14] H. Cai, Y. Xu, N. Zhu, P. He, Y. Fang, Analyst 127 (2002) 803.

[15] E. Palecek, M. Fojta, F. Jelen, Bioelectrochemistry 56 (2002) 85.

[16] B. Simmons, S. Li, V. John, G. McPherson, A. Bose, W. Zhou, J. He, Nano Letters 2 (2002) 263.

[17] T. Torimoto, M. Yamashita, S. Kuwabata, T. Sakata, H. Mori, H. Yoneyama, J. Phys. Chem. B 103 (1999) 8799.

ELSEVIER

Electrochemical detection of DNA hybridization based on DNA-templated assembly of silver cluster

Joseph Wang [a,*], Oscar Rincón [a,b], Ronen Polsky [a], Elena Dominguez [b]

[a] Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA
[b] Department of Analytical Chemistry and Chemical Engineering, University of Alcalá, Alcalá de Henares, Madrid 28871, Spain

Abstract

The growth of metals on DNA templates has generated considerable interest in connection to the design of metallic nanostructures. Here we exploit the DNA-induced generation of metal clusters for developing an electrical biosensing protocol. The new hybridization assay employs a probe-modified gold surface, and is based on the electrostatic 'collection' of silver cations along the DNA duplex, the reductive formation of silver nanoclusters along the DNA backbone, dissolution of the silver aggregate and stripping potentiometric detection of the dissolved silver at a thick-film carbon electrode. The new protocol thus combines the inherent signal amplification of stripping analysis with effective discrimination against nonhybridized DNA.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: DNA detection; Silver; Stripping analysis; DNA metallization

1. Introduction

Sequence-specific DNA detection has become a topic of substantial interest owing to its applicability to diagnosis of diseases, drug screening or forensic analysis [1]. Recent and promising activity in the field of DNA analysis has involved the use of nanoparticle-based materials. In particular, the use of oligonucleotide-functionlized gold nanoparticles and the hybridization-induced formation of DNA-linked nanoparticle networks has led to several optical [2,3] and electrochemical [4–6] DNA detection methods. The latter have relied on binding of the gold nanoparticles to the captured target, followed by their acid dissolution and an anodic stripping electrochemical detection of the dissolved metal tracer. Alternative routes for capturing metal tags have not been examined.

Here we describe a new electrochemical protocol for detecting DNA hybridization based on preparing the metal marker on the DNA itself. The use of DNA as a metallization template has evoked substantial research activity directed to the generation of conductive nanowires and the construction of functional circuits [7–9]. Such approach was applied to grow silver [7], palladium [8] or platinum [9] clusters on DNA templates. Yet, the DNA-templated assembly of metal wires has not been exploited for detecting DNA hybridization. The new detection scheme (Fig. 1) consists of the vectorial 'collection' of silver ions along the captured DNA target (d), followed by hydroquinone-catalyzed reductive formation of silver aggregates along the DNA skeleton (e), dissolution of the nanoscale silver cluster (f) and detection of the silver by a highly sensitive chronopotentiometric stripping protocol (g). The performance characteristics of the new bioassay are reported in the following sections.

2. Experimental

2.1. Apparatus

A gold disk working electrode (2 mm diameter; BAS) was used for the hybridization and silver 'collection'

---

* Corresponding author. Fax: +1-505-646-2646.
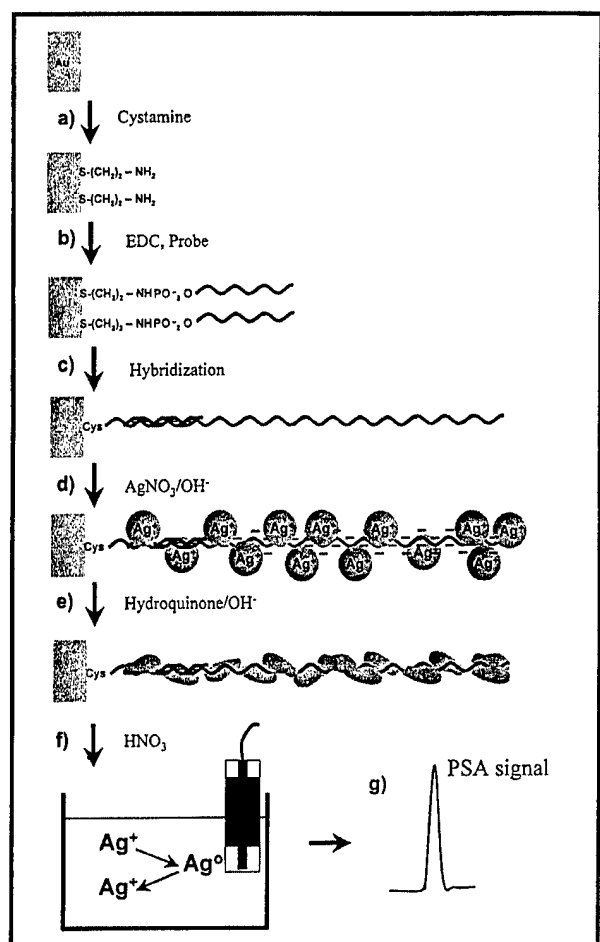 *E-mail address:* joewang@nmsu.edu (J. Wang).

Fig. 1. Schematic representation of the immobilization and analytical protocols: (a) formation of a self-assembled cystamine monolayer; (b) immobilization of ssDNA 'probe' through the 5'-phosphate groups of ssDNA by the formation of phosphoramidate bond with the amino groups of the electrode surface; (c) hybridization of the E908X-WT complementary target; (d) 'loading' of the silver ion onto the DNA (Na^+/Ag^+ ion exchange); (e) hydroquinone-catalyzed reduction of silver ions to form silver aggregates on the DNA backbone; (f) dissolution of the silver aggregates in a nitric acid (50%) solution and transfer to detection cell; (g) stripping potentiometric detection.

reactions. Potentiometric stripping analysis (PSA) of the dissolved silver was carried out with the Trace Lab potentiometric stripping unit PSU20 (Radiometer) controlled by the TAP2 software (Radiometer). A screen-printed carbon electrode (SPE) was used as the working electrode in PSA measurements. The screen-printed electrodes (SPEs) were manufactured using a semi-automatic screen printer (Model TF-100, MPM, Franklin, MA), using a carbon ink (Acheson 440B, Acheson Colloid, Ontario, CA) and alumina ceramic plates. The electrodes were cured for 1 h at 150 °C. A layer of insulator (ESL protective ink 240-5B, ESL, King of Prussia, PA) was then printed onto a portion of the conducting 'lines', exposing a rectangular (1.0 mm × 2.5 mm) working electrode area. An Ag/AgCl electrode

(CH Insturments) and a platinum wire were used as the reference and counter electrodes, respectively.

2.2. Chemicals

All solutions were prepared using deionized and autoclaved water. The following chemicals were purchased from Sigma: cystamine, 1-ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC), silver nitrate, hydroquinone and potassium phosphate monobasic and dibasic anhydrous salts. Nitric acid and hydrogen peroxide (30%) were obtained from Fisher, while sulfuric acid was received from VWR Scientific Products. Oligonucleotides were acquired from Sigma-Genosys and had the following sequences:

> Immobilized probe: 5'GAT TTT CTT CCT TTT GTT C
> Target-E908X-WT/P: 5'ATC CTG AGC TGT TGA CCA GTT ACG GCA CAG ATT CTG TTC ACG TTA CAC GGG TTG AAC AAA AGG AAG AAA ATC
> Noncomplementary target: 5' AGA CGC GCA GTG AGT CGG CCT TTG TAG ACG CAT ATG GAC CGA TTA CTA CCT CCA TTT CTA AAA GAA GGA AAA

2.3. Procedure

2.3.1. Gold electrodes pretreatment/modification

The surface modification and probe immobilization were based on an early protocol [10]. Prior to use, the gold disk electrode was soaked for 30 min in a freshly prepared piranha solution (30% hydrogen peroxide solution/70% concentrated sulfuric acid). Warning: piranha solution should be handled with extreme care. Subsequently, the electrode was polished with an alumina (0.05 μm) impregnated felt; then, its potential was reversibly cycled between 0.0 and 1.7 V (using a 0.5 M H_2SO_4 medium) until the characteristic 'gold/H_2SO_4' peak [9] was observed.

The surface modification is illustrated in Fig. 1(a) and (b). The electrode was immersed in a 2.25 mM (10 mg/ ml) aqueous cystamine solution overnight (at room temperature) allowing the gold–thiol binding. The electrode was rinsed vigorously with deionized water after each treatment.

The cystamine-modified electrodes was immersed for 6 h at room temperature in a 0.1 M EDC/0.05 M phosphate buffer (pH 7.4) solution, containing 100 μg/ ml of the ssDNA 'probe'. After this treatment, the oligonucleotides were attached through their 5'-phospate group (via the formation of phosphoramidate bond with the amino groups of immobilized cystamine molecules). The electrodes were then soaked in a 0.4 M NaOH/0.25 w/v% SDS 'wash' solution for 5 min (at 50 °C) in order to remove the nonspecifically absorbed DNA.

2.3.2. Hybridization of DNA-modified electrodes

The hybridization of the immobilized probe to the complementary nucleic acid (E908X) target was carried out in a stirred solution (0.05 M phosphate buffer, pH 7.6) containing the desired amount of DNA target. The hybridization reaction proceeded for 30 min at room temperature. Then, the electrode was soaked in a stirred phosphate-buffer solution for removing nonspecifically absorbed target DNA.

2.3.3. Silver deposition and dissolution

The DNA-template induced generation of silver nanocluster was based on an earlier procedure [7]. The electrostatic 'collection' of silver ion was proceeded by soaking the resulting DNA-modified electrode in an ammonium hydroxide (pH 10.5) solution containing 0.1 M $AgNO_3$. Metallization of the 'collected' silver was carried out (after a vigorous washing) by dipping the electrode in an ammonium hydroxide (pH 10.5) medium containing 0.05 M hydroquinone (in the absence of silver in the solution). Each of the above processes was carried out for 15 min in the dark.

The dissolution of the metallic silver aggregate was carried out by spreading a 5 µl drop of nitric acid (50%) onto the inverted surface for 5 min. The acid drop containing the dissolved silver ions was transferred to a 0.1 M HNO_3/0.1 M KNO_3 measuring solution.

2.3.4. Chronopotentiometric stripping measurements

Each experiment was carried out with a 'fresh' SPE strip, and included an initial background evaluation followed by the actual detection of the dissolved silver. PSA measurements were performed by treating the surface at +1.2 V for 3 min followed by a 1 min deposition of the silver at −0.5 V using a stirred 1 ml 0.1 M HNO_3/0.1 M KNO_3 solution. Subsequent stripping was performed in a quiescent solution using an applied anodic current of +3.0 µA. The stripping curve data were filtered and baseline corrected with the TAP2 software.

3. Results and conclusions

The new protocol relies on DNA-template induced generation of conducting nanowires as a mode of capturing the metal tag. The DNA probe was covalently attached to the self-assembled cystamine monolayer through a 1-ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC) linker. The new concept is illustrated here in connection to a reductive formation of silver aggregates along the DNA skeleton and potentiometric stripping detection of the dissolved silver. Electrochemical stripping DNA detection of a silver tracer was accomplished previously in connection to captured nanoparticle tags [5,6], but not through metallization of the DNA backbone.
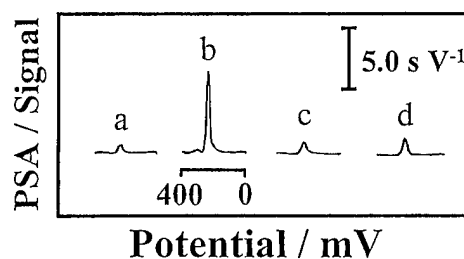

Fig. 2. Chronopotentiometric stripping hybridization response to: (a) 0 µg/ml of E908X-WT complementary target; (b) 1 µg/ml of E908X-WT complementary target; (c) 20 µg/ml of noncomplementary target; (d) 100 µg/ml of noncomplementary target. Conditions: hybridization time, 30 min in a 0.05 M phosphate-buffer solution; stripping current, +3.0 µA; deposition at −0.5 V for 1 min using a 0.1 M HNO_3/0.1 M KNO_3 medium; SPEs pretreated at +1.2 V for 3 min.

The new assay couples the high sensitivity of electrochemical stripping detection of metal tracers with effective discrimination against nonhybridized DNA. Such attractive performance was illustrated for the detection of DNA segments related to the BRCA1 breast cancer gene. Fig. 2(b) displays the silver hybridization stripping response for a 1 µg/ml DNA target. A well-defined and sharp chronopotentiometric silver signal is observed ($E_p = 0.22$ V). Substantially smaller signals are observed for solutions containing 20- and 100-fold excess (20 and 100 µg/ml) of noncomplementary DNA (Fig. 2(c) and (d), respectively). Such minimization of contributions of nonhybridized DNA is attributed to the blocking by the self-assembled cystamine layer. (The surface packing was indicated from the nearly complete suppression of a ferrocyanide cyclic voltammetric peak; not shown.) Indeed, the response for the 100-fold excess of noncomplementary DNA is only slightly larger than that observed in the absence of nucleic acids (Fig. 2(a)). The small background contribution (in the absence of nucleic acids) reflects the 'collection' of the silver ions on the short (19 bp) probe. Practical applications commonly involve longer DNA target and hence would lead to smaller relative background contributions. The use of neutral PNA probes – that do not promote electrostatic
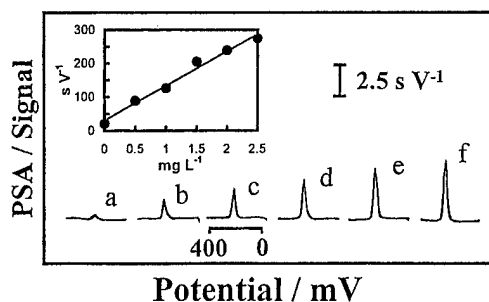

Fig. 3. Chronopotentiometric stripping responses for increasing concentrations of the E908X-WT complementary target in 500 ng/ml increments (b–f), along with the response in the absence of the target (a). Inset figure shows the calibration curve. Conditions as in Fig. 2.

'collection' – would eliminate such background signal (while offering effective mismatch discrimination).

Such DNA-metallization based bioassay results in a well-defined concentration dependence. Fig. 3 displays typical chronopotentiograms for solutions containing increasing target concentrations in 500 ng/mL steps (b–f), along with the response for the blank solution (a). Well-defined silver stripping peaks are observed in connection to these low target concentrations. The peak area increases linearly with the target concentration. The resulting calibration plot (shown in the inset) is highly linear over the entire 500–2500 ng/ml range (slope of 1.03 ms ml/ng; $r = 0.992$).The response for a 250 ng/ml target DNA solution indicates a low detection limit of around 100 ng/ml, which corresponds to 5 ng in the 50 μl hybridization solution (not shown; 10 min deposition). Such detection limit is similar to that obtained in bioassays based on captured gold nanoparticle tags [4]. Further lowering of the detection limit is expected in connection to longer targets, longer hybridization times or deposition periods, via catalytic silver precipitation (onto the silver cluster), or by 'collecting' the silver onto multiple branches of a secondary dendritic probe (of sandwich assays). A series of six repetitive measurements of the 1.0 μg/ml target solution was used for estimating the precision (not shown; conditions, as in Fig. 2). This series yielded a mean peak area of 131 ms and a relative standard deviation of 24%. Such signal variations reflect the reproducibility of the surface modification, hybridization, silver 'collection'/dissolution, and stripping steps.

4. Conclusions

We have demonstrated for the first time the use of DNA metallization for electrochemical monitoring of DNA hybridization. The new protocol couples the high sensitivity of electrochemical stripping transduction with an effective discrimination against excess of unwanted constituents. The DNA-template induced generation of other metal tracers could found useful for nucleic acid electrical assays. We are currently examining a similar use of a platinum tracer in connection to ultrasensitive catalytic-adsorptive stripping detection. Other improvements involving PNA or dendritic probes, as well as catalytic metal enlargement are also being explored.

References

[1] J. Wang, J. Nucleic Acids Res. 28 (2000) 3011.
[2] T.A. Taton, C.A. Mirkin, R.L. Letsinger, Science 289 (2000) 1757.
[3] J.J. Storhoff, R. Elghanian, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 120 (1998) 1959.
[4] J. Wang, D. Xu, A.N. Kawde, R. Polsky, Anal. Chem. 73 (2001) 5576.
[5] J. Wang, R. Polsky, D. Xu, Langmuir 17 (2001) 5739.
[6] H. Cai, Y. Wang, P. He, Y. Fang, Anal. Chim. Acta 469 (2002) 165.
[7] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 391 (1998) 777.
[8] J. Richter, R. Seidel, R. Kirsch, M. Mertig, W. Pompe, J. Plashke, H. Schackert, Adv. Mater. 12 (2000) 507.
[9] M. Mertig, L.C. Ciacchi, R. Siedel, W. Pompe, A. de Vita, Nano Lett. 2 (2002) 841.
[10] C. Fan, G. Li, Q. Gu, J. Zhu, D. Zhu, Anal. Lett. 33 (2000) 1479.